

Report on Rennet-Volume 1

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R E P O R T

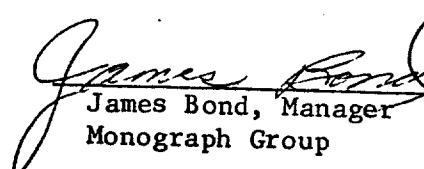
RENNET  
(RENNIN)  
ACCESSION NO. 522  
CAS. REG. NO. PM9001983

Submitted to:  
GRAS Review Branch (BF-335  
Bureau of Foods  
Food and Drug Administration  
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## SUMMARY

Most proteolytic enzymes will clot milk but on further contact will dissolve the curd hydrolysis of the insoluble proteins to peptides and amino acids. One of the oldest uses of an enzyme is that of rennet for coagulating milk in cheese-making. Rennin is unique among the milk-curdling enzymes; after the clotting of milk, there is no further rapid or extended sensitive action on the curd. The mechanism of curd formation is speculated to involve limited proteolysis of k-casein by rennin-forming soluble glycopeptides and paracasein. The paracasein precipitates and leads to the precipitation of other casein fractions which have been exposed to calcium ions released as a result of the k-casein hydrolysis (99a).

In the 1972 Comprehensive GRAS study, 281,371 lbs. of rennet were reported used in the food categories of milk, cheese, frozen dairy, and gelatin, with the greatest percentage being used in the manufacture of cheese and gelatin (218b).

A search for substitutes for rennet for milk coagulation and cheese-making has been under way for many years. In certain countries (such as India) religious requirements make a vegetable rennet desirable because the source of commercial rennet is the stomach mucosa of young calves. While there is a continuing increase in demand for cheese (and therefore rennet), there is at the same time a decrease in the number of young calves being slaughtered, and hence there is a shortage of rennet (116).

In the limited amount of information available for this review it was found that pre-curdling of milk gave favorable results in infants suffering from dystrophy, atrophy, loss of appetite, pylorospasm and other diseases which were accompanied by vomiting (116).

In dogs, a 1% rennet solution N/10 HCL produced perforated ulcers more rapidly than did N/10 HCL alone (73).

It was found that acid-treated egg whites can be used to separate hog pepsin and calf rennin; further separation of the calf rennin shows that the calf preparation contains two fractions which were distinctly different. Phenyl sulphite esters may be suitable substrates for assaying the activity of the rennin preparations (98, 135).

Rennin will not clot dephosphorylated casein; however, if the casein is enzymatically rephosphorylated, the clotting activity of rennin is restored (19).

In human subjects there is a significant correlation between abnormally high concentrations of rennin inhibitor and catatonic schizophrenia (146)

The injection of rennet solutions into rabbits once weekly for a period of 4 weeks produced an antirennin serum which markedly diminished the rate of rennin clot formation (211).

Chemical Information

I. Nomenclature (166a, 207a, 222)

A. Common Names

Rennin

Chymosin

Rennase

Abomasal enzyme

German: lab Ferment

B. Chemical Names

No chemical names were encountered in the literature searched.

C. Trade Names

P/L Rennet Extracts, Liquid

P/L Rennet Powder

Junket<sup>1</sup>

D. Chemical Abstracts Unique Registry Number

PM 9001 - 98 - 3

II. Empirical formula

No empirical formula was encountered in the literature searched

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<sup>1</sup>A dessert preparation containing rennet, a product of Salada Foods Inc., Woburn, Mass.

### III. Structural formula

No structural formula was encountered in the literature searched.

### IV. Molecular Weight (93a, 207a)

Prorennin: 50,000

Rennin Fractions: 30,700 - 36,000

Fungal Rennin: 34,000 - 39,000

### V. Specifications (197)

#### A. Chemical

Rennin has: 17 - 50 % of rennet extract activity

9.0 % H<sub>2</sub>O of crystallization

0.98% ash

0.89% S

#### B. Food Grade

No food grade specifications were encountered in the literature searched.

#### C. Official Compendia

Rennet was not encountered in the official compendia searched.

### VI. Description (197,207a)

#### A. General Characteristics

Rennin, a dried extract of rennet: yellowish powder;

grains or scales; peculiar, not unpleasant odor; characteristic, slightly salty taste.

#### B. Physical Properties

Water solutions - not stable

Partially soluble in dilute alcohol

Soluble in dilute salt and dilute HCL

Nondialyzable

pH of aqueous solution - 5.8 (acceptable range 5.3 - 6.3)

Precipitable with ammonium sulfate, organic solvents, acetone, and isopropanol.

Destroyed by heating for 5 minutes at 60°C

Most stable at pH 4.5

Isoelectric point: pH 5.5

Dried; resists heating at 100°C for 4 hours or more

Slightly hydroscopic

Strongly affected by ultraviolet (sunlight)

Crystalline rennin: m.p. 225-227°C (decomp.)

Electrophoresis - 1 peak with same mobility as  $\alpha$ -globulin

1) -and Table 1. Rf-values of crystalline rennin and pepsin are as follows;

SOLVENT	RF-VALUE	
	rennin	pepsin
n-butanol-pyridine-water pH 6.3 . . .	0.26	0
Collidine- water, pH 6.5 . . . . .	0.83	0.05
Phenol-NH <sub>4</sub> OH, pH 6.3 . . . . .	0.10, 0.21, 0.81	0.63
80% Ethanol, pH 5.9 . . . . .	0.85	0.63
90% Methanol, pH 6.0 . . . . .	0.83	0
iso-amyl alc.-NaCitrate pH 5.9 . . . .	0.20	0.20

2) The electrophoretic patterns of rennet-extract, precipitated crude rennin and crystalline rennin are obtained.

### C. Stability

No reference to studies of the stability of rennin in containers was found in the literature searched. However, dried rennin has been found to be stable for at least 4 hours at 100°C (197). Masks and protective clothing are recommended when handling fungal rennet (58).

### VII. Analytical Methods

Material:

Rennet

Title:

A micro-method for the quantitative estimation of Rennets and other proteolytic enzymes (184).

Principle Determination Step:

Measurement of the diameter of the zones of precipitation or of the transparent zones formed with rennin using calipers.

Principle Separation Technique:

No separation of the rennet was performed after purchase.

Principle Analytical Reagent:

10% whole casein

Moiety Measured:

Indefinite

Sensitivity:

0.003 ml 1% w/v solution of fungal rennet.

Substrate:

1% caseinate + 10-15 mM  $\text{Ca}^{2+}$ .

Material:	Rennet
Title:	The Detection of Rennin in Cheese with the Passive Indirect Hemagglutination Test (79).
Principle Determination Step:	Hemagglutination inhibition
Principle Separation Technique:	None
Principle Analytical Reagent:	Anti-rennin serum
Moiety Measured:	Indefinite
Sensitivity:	1 - 2 mg of rennin/ml, 3 mg of rennin/g cheese.
Substrate:	Rabbit anti-rennin serum

#### VIII. Occurrence (28a, 58, 229, 273)

##### A. Plants (extracts showing rennet or rennin like activity)

*Endothia parasitica*

*Ficus coagulans*

*Withania coagulans*

*Mucor pusillus*

*Bacillus cereus*

##### B. Animals

*Abomasum of young cows*

*Abomasum of lambs*

*Other Herbivorous animals*

*Older bovines*

##### C. Synthetics

No occurrence or levels in synthetics could be found in the

The curdling time of hydrochloric acid maceration of the stomachs of calf and human fetuses was studied in an experiment to determine the appearance of rennin during the fatal stages of life.

In the calf aged 50 to 135 days, it was found that curdling time decreased as age increased; the same pattern was observed in hydrochloric acid macerations of the stomachs of 6 to 8 month-old human fetuses (243).

Anti-rennet serum was obtained from rabbits injected once weekly over a period of 4 weeks with 5 cc or a 1% rennet solution. A decrease in the curdling action of rennet was observed when anti-rennet was added to the milk media. Tanic acid also decreased the coagulation effect of rennet when studied under the same experimental conditions (211).

In an experiment studying the digestion of milk in humans, it was found that the addition of rennin to milk had no effect in regard to the proportion of nitrogen liberated from its peptide linkage, the time in which the maximum liberation was accomplished after ingestion, or the amount of speed of milk digestion (92).

Favorable results in 130 clinically observed infants suffering from dystrophy, atrophy, loss of appetite, pylorospasm and other forms of milk dyspepsia were obtained by precurdling cow's milk with peginin before feeding (116).

During an in vitro study of enzyme-induced ulcer production in dog intestines, 0.1% rennin N/10 HCL produced perforated ulcers in 114 minutes, whereas N/10 HCL alone caused perforation in 174 minutes. This data demonstrated that rennin had marked digestive effect on the intestinal mucosa but not as great an effect as pepsin (73).

Specific absorption studies in calf rennin were conducted to obtain further proof that the activities of pepsin and calf rennin can be completely separated by absorption in acid-treated egg white and that the calf preparation contains two fractions which can be separated from each other without destruction of the milk curdling ability of either one (98).

A study of synthetic peptide and ester substrates was done to determine if they might be suitable substrates for assaying the activity of rennin preparations; this study revealed that rennin hydrolyzes sulphite esters such as phenyl sulphite ester and some N-substituted imidazole compounds such as bensoyl and imadazole. Phenyl sulphite esters may be suitable substrates for assaying the activity of rennin preparation (135).

Studies conducted on the action of rennin on casein and the nature of clotting demonstrated that whole protein required more calcium ions for coagulation than did alpha-casein, which suggested that rennin may release imidazole or phenolic groups from intramolecular linkage (130).

Another study on the mechanism of rennin action on the clotting of casein showed that the clotting was not affected when H<sub>2</sub>S and p-chloromercuribenzoate was used. When amino groups were substituted, rennin did not clot casein, but when dephosphorylated casein was enzymatically rephosphorylated, the clotting activity of rennin was restored (19).

Serum rennin inhibitor studies were conducted in 96 hospitalized male schizophrenic patients. A significant correlation was found between an abnormally high rennin inhibitor titer and catatonic schizophrenia in those patients exhibiting classic states of stupor or excitement. The mood, the affective and intellectual status, changes in weight or libido, and the chronicity of the illness did not correlate with the enzymatic disturbance (146).

In a review article entitled "Rennet - Past, Present and Future", it is suggested that rennet be divided into 3 classes designated A, B, and C. Type A would consist of true rennet derived from stomachs of suckling calves; type B would consist of rennet derived from the stomachs of older bovines; type C would consist of rennet derived from other herbivorous mammals, whose milk would be used for cheese-making (58).

RENNET

Bibliography

\*\* Article to be found in summary

\* Article to be found in text

## RENNET

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